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Production and Sensory Evaluation of Non-Alcoholic Wine from Sugarcane and Tiger Nut Blend Using *Saccharomyces cerevisiae*

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ABSTRACT
This study produced a dealcoholic wine from sugarcane and tiger-nut blend using *Saccharomyces cerevisiae.* Sensory and physiochemical properties were assessed and the effect of fermentation (60days) on microbial profile and pH of non-alcoholic wine were evaluated. Dealcoholization process was done by distillation at 70 ^{0}C for 5hrs. Three blend of wine ratios (sugar cane juice : tiger-nut milk), grouped as sample A (5:1), sample B (5:2) and sample C (6:0), were predominanted by *Lactobacillus spp* (28.6%) and *Bacillus spp*(42.9%), though below significant threshold. Alcoholic content of wine blends before dealcoholization showed that sample C (6:0) has the highest alcoholic content of 13.39%, followed by sample A (10.11%) and sample C (6:83%) was the least. After dealcoholization, sample A and B have the same alcoholic content of 0.13% each and sample C (6:0) was 0.01%.. Dealcoholization decreased the pH of all the wine blends (A, B & C) by 19.1%, 17.7%. & 30.3% respectively and altered the taste and appearance of the wines significantly. In conclusion, Sample B was the best wine based on sensory evaluations. Perhaps, nutritional richness of tiger-nut milk at adequate ratio, directly improves the sensory acceptance of dealcoholiced wine. This study recommends that wine of sugarcane and tiger nut blend should be fermented at temperature not exceeding 24^{0}C to 27^{0}C to evade failed fermentation due to hindered yeast growth.

Keywords: Non-alcoholic, wine, sugarcane, tiger-nut, *saccharomyces cerevisiae*

INTRODUCTION

Wine is among the popular alcoholic beverages traditionally produced through fermentation of carbohydrate substrates of plant origins using yeast. It is technically a product of naturally or induced biochemical transformation of these plant (fruits cereals, grapes, nuts, saps, etc) extracts followed with processing of clarification, bottling, aging and flavoring [1]. The quality of every wine depends on the careful methods at each stage. Sugarcane-tiger nut juice extract is a new wine substrate blend assessed by this study. Sugarcane as a rich source of sucrose serve as a readily carbon source for efficient bioconversion to ethanol [1] comparable to other substrates such as wheat, rice, maize and sorghum. *Cyperus esculentus lativum* commonly known asTiger nut is a crop under the family of sedge [2]. Flavor and health potencies were the intent of incorporating Tiger nut as a blending agent due to their high fiber content, , vitamins, minerals modulate blood pressure and sugar level [3, 4].

Most alcoholic wines are fermented with Saccharomyces cerevisiae, eiher in pure form or in consortium of other yeast. Initial fermentation phrase are dominated by non-*saccharomyces* species such as *Candida sp and Hanseniaspora sp* that have slight but significant fermentative impact on the ethanol quality of the product [5, 6, 7], and they account for 5 -6% (v/v) ethanol content within 2-3days, and rapidly decline thereafter [8, 9]. However, most *Saccharomyces* related species (*Schizosaccharomyces, Brettanomyces, Kluyveromyces Saccharomycodes, Zygosaccharomyces, and Torulaspora* sp) dominates the later fermenting and aging stage.

Due to increasing campaign on health, religious and moral issues recently, non-alcoholic beverages are gaining tremendous popularity. The term non-alcoholic beverages refers those beverages that have less than 0.5% alcoholic content by volume to zero percent(0%) which is known as alcohol-free. All methods to dealcoholize beverages are targeted to achieve this range of 0 - 0.5% [10]. Two major approaches to dealcoholize wine with minimal heat are by vacuum distillation (low boiling point below atmospheric pressure) and reverse osmosis (using semi permeable membrane in reverse osmosis) to filter the alcohol. These two methods, unlike the conventional thermal distillation, water and organic compounds are return back into the residues. The benefits of non-alcoholic wine are not limited to

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decreased risk of heart disease, cholesterol level and stroke.

MATERIALS AND METHOD

Sample Collection

Sugarcane and Tiger nut were purchased from market in Lapai, Niger state, and samples were transported to Ibrahim Badamasi Babangida University Lapai, Applied microbiology and quality assurance laboratory and refrigerated until used.

Preparation of Inoculum Starter Culture

Pure colonies of S. *cerevisiae* obtained from repository of Applied Microbiology laboratory, Ibrahim Badamasi Babangida University, and verified by culturing with potatoes dexterous broth (PDB) in 250ml Erlenmeyer conical flask and incubate at ambient temperature for 24hrs with relevant biochemical assay. Verified Isolates were primed in 3 to 6hrs PDB culture and were adjusted to cell concentration of 10^6cfu/mL and stored at 4^0C prior to inoculation.

Preparation of Substrate Blend (Sugarcane Juice and Tiger Nut Milk)

Sugarcane juice and tiger nut milk extracts were obtained by sorting and washing the samples thoroughly, chopped (~1cm^2) before mincing with industrial electric blender for 20mins to achieve smooth slurry. The two separate slurries were sieved in muslin bag to obtain the sugarcane juice and tiger nut milk.

Fermentation and Experimental Design

Three blend ratios of sugarcane juice (SCJ) to tiger nut milk (TNM) mixture of 6:0 (600ml + 0ml), 5 : 1(500ml + 100ml) and 5:2 (500 + 100ml) were separately placed in 2000ml round bottom flask and labelled as A, B and C respectively. Each flask was steam sterilizer at 121^0C for 15 minutes and cooled to room temperature. Starter culture (50ml) was added to the flasks and allowed to stand for 60days at microaerophilic conditions. periodic degassing and agitating of the flasks (fermenting system) were done at 2 days intervals. Clarification was by siphoning supernatant of wine sediments prior to sensory evaluation and dealcoholization (distillation at 80^0C for 5hours).

Specific Gravity

Specific gravity was determined measuring 50ml of the sample into volumetric flasks at 20^0C; and hydrometer was dipped into it to determine the specific gravity (with appropriate temperature correction factor). The percentage alcohol content, calories and attenuation were then calculated based on specific gravity chart [12].

Microbial Analysis

Microbial assays (isolation, characterization and enumeration) were done on samples collected at 15days fermentation intervals in accordance with the methods of Cowan and Steel [11].

Sensory Evaluation

The sensory evaluation of the wines were evaluated by 50 panelists (trained and untrained) drawn from staffs and students of IBB University Lapai community using the 9-point hedonic scale.

Statistical Analysis

Data generated from the sensory evaluation were subjected to ANOVA, Chi – square and Duncan's Multiple Range Test using SAS statistical software (Version 8, SAS institute, Cary, NC, USA) at 95% confidence level and SPSS software version 20 of 2014.

RESULT AND DISCUSSION

Bacterial prevalence after 60days of fermentation were in the decreasing pedigree of *Bacillus* sp (42.9%), *Lactobacillus* sp (28.6%), *Corynebacterium* sp (14.3%) and *Micrococcus varians*(14.3%) has the least percentage (Fig 1). While Yeast isolates were dominated by *Saccharomyces* sp (60%) , *Pichia* sp and *Kluveromyces* sp were 20% each (Fig 2). Nutritional richness of sugarcane and tiger nut juice makes a good culture media for microbial growth. This account for the obvious high prevalence of yeast (20 - 60%) and opportunistic bacteria (14.3 - 42.9%) among the wines. Similar report have been documented [13, 6, 7] with high prevalence of both yeast and bacterial agents from alcoholic wine. There was no significant different ($P<0.05$) on bacterial load (1.79×10^4cfu/ml - 1.9×10^4cfu/ml) among the three blends of wines after 60days fermentation; contrarily to yeast load (1.0×10^6 cfu/ml - 4.0×10^6 cfu/ml) that significantly differ among the three blends of wine (Fig. 3). Higher bacterial load observed in wine without tiger nut milk; supports the fact that some natural inhibitors delays the log phrase of microbial kinetic in the

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fermenting system. Other dimension to this explanation is predicated on the alteration of optimum pH of wine blended with tiger nut, which probably slows down the microbial proliferation rate. In a similar study by Towobola[14], blending of honey wine with coconut milk alters the optimum pH, which correlates to significant adverse affect on microbial growth.

Sensory evaluation scores of three blend of sugarcane-tiger nut alcoholic wines, showed that only the B(5:2) blend has a significant poor acceptability rating (4.56 - 5.55) when compared to the blends of A(5:1) and C(6:0) with scores range of 1.85 - 3.65 and 3.65 - 4.20 respectively. Post Hoc Duncan multiple range test indicted all sensory parameters (aroma, taste, appearance and consistency) of A (5:2) for contributing to the poor acceptability rating (Table 1).

Dealcoholization by distillation significantly ($P<0.05$) altered the pH of sample C from 4.08 to 5.86, but samples A and B were not significant ($P>0.05$) at the range of 3.87 - 4.89 (Figure 4). Interestingly, pH of wines blended with tiger nut milk were relatively stable after dealcoholization, unlike the control that was significantly ($P<0.05$) altered. This phenomenon is not unconnected to the microbial (yeast) kinetics [14, 15, 16] responses to the inhibitory molecules in tiger nuts.

After 5hrs of distillation , more than 95% reduction in alcoholic content from 13.39%v/v to 0.01%v/v was achieved (figure 5), and this significantly ($P<0.05$) reduced the titratable acidity of the three wine blends (Figure 6). Significant ($P<0.05$) downward alterations of total titratable acidity of wines are traceable to the escape of volatile organic acids during thermal distillation (70^0C). Sample B (5:2 blend) was rated the overall best with average hedonic score of 1 point, followed by sample C with 2.25 points and sample B had 3.0 points (Figure 7). Surprisingly, dealcoholization enhanced the overall sensory evaluation rating of wines by more than 50%, though; aroma was the prominent parameter that was adversely impacted. Conclusively, non-alcoholic wine of sugarcane and tiger nut blended at the ratio of 5:2 has the best sensory quality rating. Perhaps, presences of more quantity of phenolic compounds [17], and flavour agents in A(5:2) than in B (5:1) explains the variation. This study was done under room temperature thus recommends that non-alcoholic wine of sugarcane and tiger nut should be fermented at temperature range of 24^0C to 27^0C to limit chances of failed fermentation.

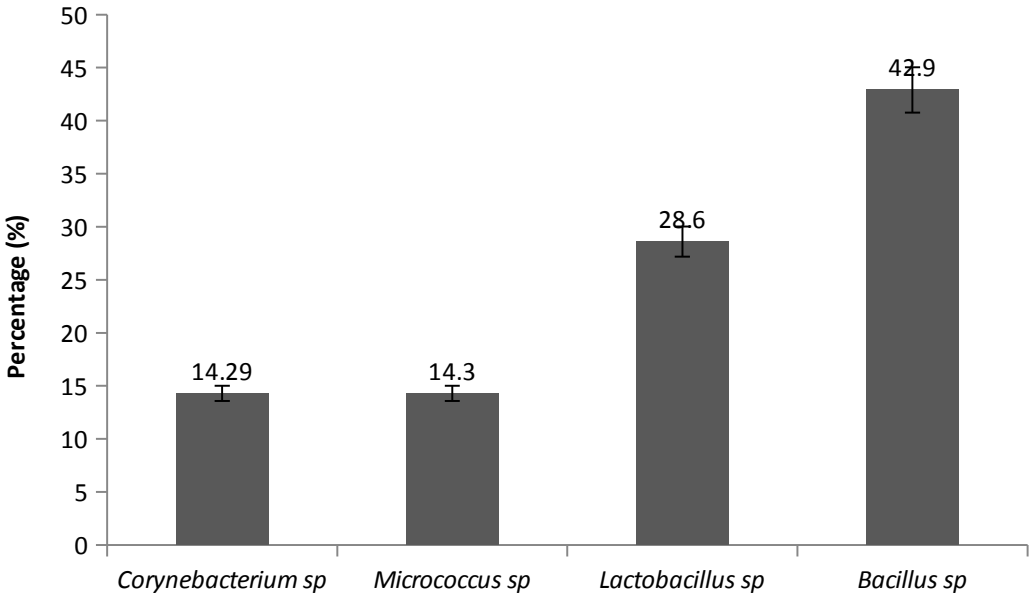

Figure 1: Prevalence of bacterial isolates from wine after 60 days fermentation
NB: Bars bearing different alphabets are significantly different ($p<0.05$), n = 3

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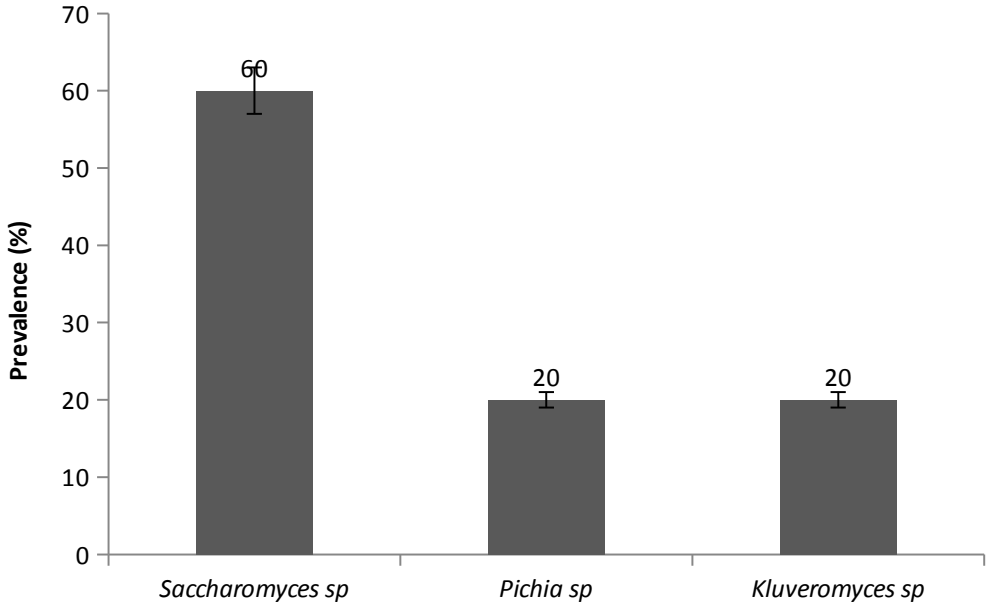

Figure 2: Prevalence of Yeast Isolates from Wine after 60 Days Fermentation.

NB: Bars bearing different alphabets are significantly different ($p<0.05$), n = 3

Table 1: Sensory Evaluation Analysis of Alcoholic Blends Sugarcane-Tiger-nut Wines

Parameter	Sample A	Sample B	Sample C
Aroma	$3.65^{a} \pm 1.13$	$5.50^{b} \pm 2.82$	$3.65^{a} \pm 2.56$
Taste	$3.00^{a} \pm 2.45$	$4.95^{b} \pm 2.89$	$3.90^{c} \pm 1.37$
Appearance	$1.85^{a} \pm 1.84$	$4.56^{b} \pm 2.66$	$4.20^{b} \pm 2.89$
Consistency	$2.85^{a} \pm 2.32$	$5.55^{b} \pm 2.87$	$3.75^{a} \pm 2.69$
Overall	$3.74^{a} \pm 1.94$	$5.14^{b} \pm 2.81$	$3.87^{a} \pm 2.38$

Mean values having the different alphabet are significant different. ($p<0.05$) across the table; n = 50

Key: Sample A (5 : 1) = 500ml of sugarcane juice + 100ml of tiger nut juice extract
Sample B (5 : 2) = 500ml of sugarcane juice + 200ml of tiger nut juice extract
Sample C (6 : 0) = 600ml of sugarcane juice + 0ml of tiger nut juice extract

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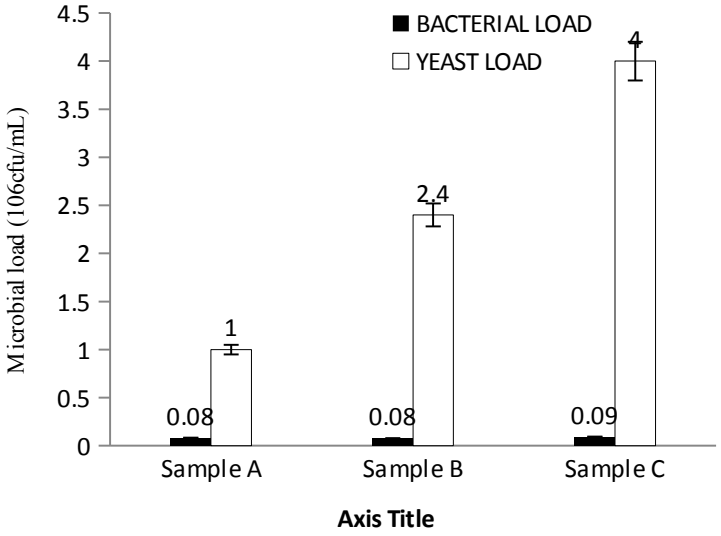

Figure 3: Microbial load of Different Wine Blends after 60 Days Fermentation

NB: Bars bearing different alphabets are significantly different ($p<0.05$) ; n = 50
Key: Sample A (5 : 1) = 500ml of sugarcane juice + 100ml of tiger nut juice extract
Sample B (5 : 2) = 500ml of sugarcane juice + 200ml of tiger nut juice extract
Sample C (6 : 0) = 600ml of sugarcane juice + 0ml of tiger nut juice extract

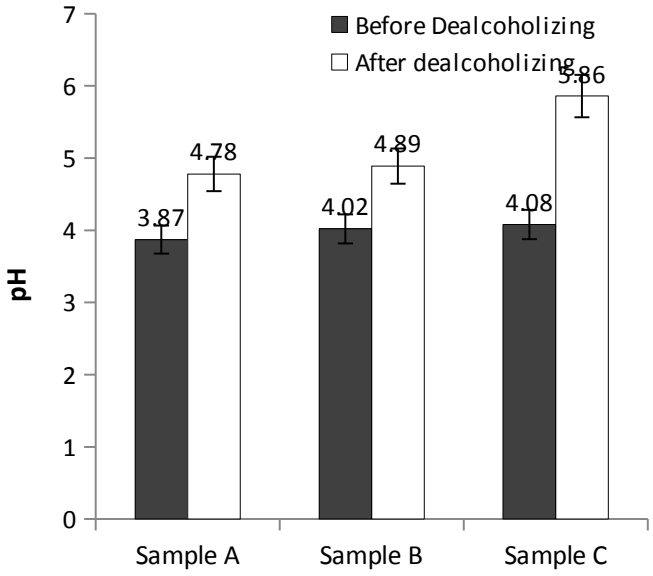

Figure 4: Effect of Dealcoholization on the pH of Different Wine Blend after 60 Days

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NB: Bars bearing different alphabets are significantly different ($p<0.05$) ; n = 50
Key: Sample A (5 : 1) = 500ml of sugarcane juice + 100ml of tiger nut juice extract
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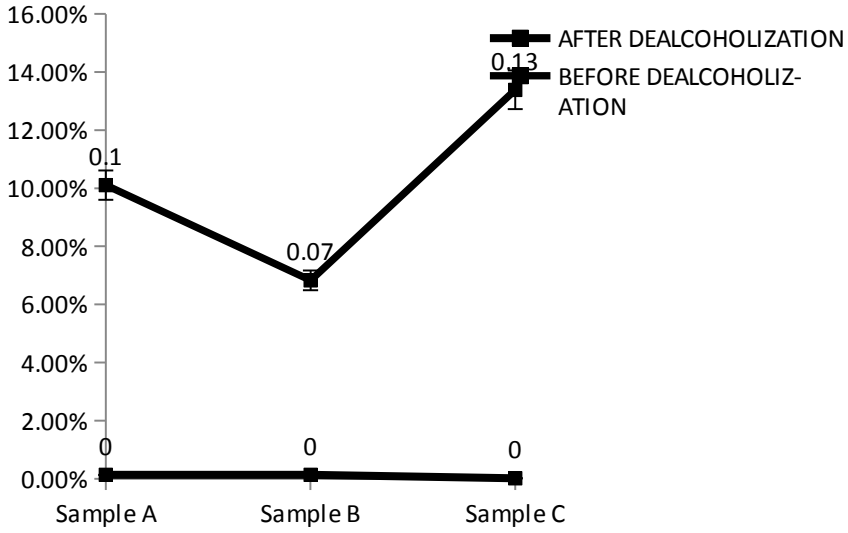

Figure 5: Alcoholic content (%) of dealcoholized wine by controlled distillation after 5hrs

NB: Bars bearing different alphabets are significantly different ($p<0.05$)
Key: Sample A (5 : 1) = 500ml of sugarcane juice + 100ml of tiger nut juice extract
Sample B (5 : 2) = 500ml of sugarcane juice + 200ml of tiger nut juice extract
Sample C (6 : 0) = 600ml of sugarcane juice + 0ml of tiger nut juice extract

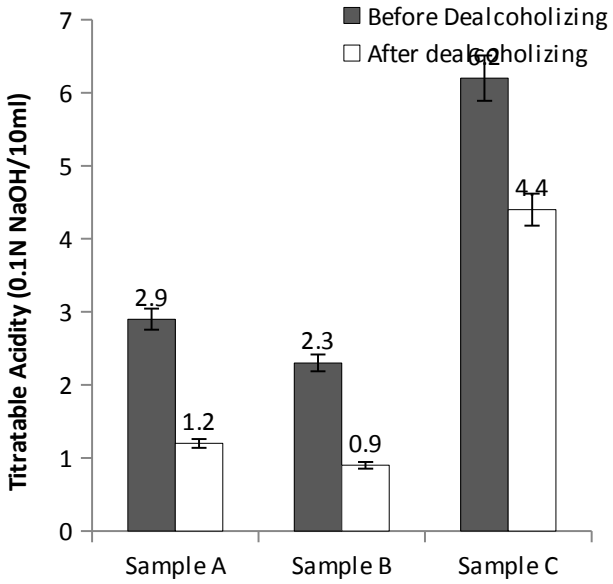

Figure 6: Effect of dealcoholization on the wine Titratable acidity

NB: Bars bearing different alphabets are significantly different ($p<0.05$)

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Key: Sample A (5 : 1) = 500ml of sugarcane juice + 100ml of tiger nut juice extract
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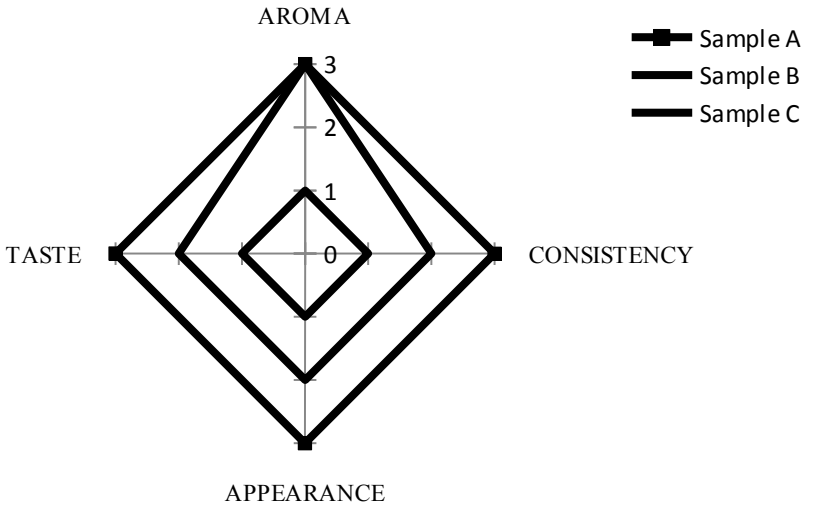

Figure 7: Acceptance rating of different dealcoholized wine based on 7-point hedonic scale
Key: Sample A (5 : 1) = 500ml of sugarcane juice + 100ml of tiger nut juice extract
Sample B (5 : 2) = 500ml of sugarcane juice + 200ml of tiger nut juice extract
Sample C (6 : 0) = 600ml of sugarcane juice + 0ml of tiger nut juice extract

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